



---

Morse, Christopher I ORCID logoORCID: <https://orcid.org/0000-0002-5261-2637>, Bostock, Emma L ORCID logoORCID: <https://orcid.org/0000-0001-9340-8459>, Twiss, Harriet M, Kapp, Laura H, Orme, Paul and Jacques, Matthew F (2018) The cardiorespiratory response and physiological determinants of the assisted 6-minute handbike cycle test in adult males with muscular dystrophy. *Muscle and Nerve*, 58 (3). pp. 427-433. ISSN 0148-639X

---

**Downloaded from:** <https://e-space.mmu.ac.uk/620492/>

**Version:** Published Version

**Publisher:** Wiley


**DOI:** <https://doi.org/10.1002/mus.26146>

**Usage rights:** Creative Commons: Attribution-Noncommercial 4.0

Please cite the published version

<https://e-space.mmu.ac.uk>

# THE CARDIORESPIRATORY RESPONSE AND PHYSIOLOGICAL DETERMINANTS OF THE ASSISTED 6-MINUTE HANDBIKE CYCLE TEST IN ADULT MALES WITH MUSCULAR DYSTROPHY

CHRISTOPHER I. MORSE, PhD <sup>1</sup>, EMMA L. BOSTOCK, PhD,<sup>1</sup> HARRIET M. TWISS, MSc,<sup>1</sup> LAURA H. KAPP, MSc,<sup>1</sup> PAUL ORME, BSc,<sup>2</sup> and MATTHEW F. JACQUES, MSc<sup>1</sup>

<sup>1</sup>Health, Exercise and Active Living Research Centre, Department of Sport and Exercise Sciences, Manchester Metropolitan University Cheshire, Crewe, CW1 5DU, UK

<sup>2</sup>The Neuromuscular Centre, Winsford, Cheshire, UK

Accepted 12 April 2018

**ABSTRACT:** *Introduction:* The assisted 6-minute cycle test (A6MCT) distance was assessed in adults with muscular dystrophy (MD). *Methods:* Forty-eight males, including those with Duchenne MD (DMD), limb-girdle MD (LGMD), fascioscapulohumeral MD (FSHD), and Becker MD (BMD), as well as a group without MD (CTRL), completed handgrip strength (HGS), lung function [forced expiratory volume in 1 second (FEV<sub>1</sub>) and forced vital capacity (FVC)], body fat, and biceps thickness assessments. During the A6MCT, ventilation (VE), oxygen uptake (VO<sub>2</sub>), carbon dioxide (VCO<sub>2</sub>), and heart rate (HR) were recorded. *Results:* A6MCT and HGS were lower in MD than CTRL subjects. FEV<sub>1</sub>, FVC, and biceps thickness were lower in MD than CTRL; lower in DMD than BMD, LGMD, and FSHD; but were not different between BMD, LGMD, and FSHD. A6MCT correlated with HGS, FEV<sub>1</sub>, FVC, body fat, VO<sub>2</sub>, VCO<sub>2</sub>, HR, and VE ( $r = 0.455\text{--}0.708$ ) in pooled BMD, LGMD, and FSHD participants. *Discussion:* A shorter A6MCT distance in adult males with MD was attributable to HGS and lung function. The A6MCT is appropriate for assessment of physical function in adults with MD.

*Muscle Nerve* 58:427–433, 2018

**M**uscular dystrophy (MD) is a heterogeneous group of neuromuscular disorders characterized by a genetic predisposition to an absence or reduction in proteins within and around the sarcolemma.<sup>1</sup> Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), limb-girdle muscular dystrophy (LGMD),<sup>2,3</sup> and facioscapulohumeral muscular dystrophy (FSHD)<sup>4</sup> all have distinct loci of impairments within the proteins of the sarcoglycan complex, which are reflected by distinct functional impairments in terms of location and severity of progression.<sup>1</sup> They

are, however, all similar in that they are associated with a loss of physical function.<sup>5</sup> Ambulatory status and walking speed are frequently used as indicators of progression and severity of various MDs.<sup>6–8</sup> The association between timed walking tests and the progression of numerous neuromuscular conditions makes them an essential, yet simple means of monitoring and screening.<sup>9</sup> Within MD, the 6-minute walk test (6MWT) is advocated based on its reliability, sensitivity, and approximation to numerous physiological outcome measures as well as its position as one of the best single measures of independence and function.<sup>6,7</sup> Although suitable for the early years of DMD, and for some ambulatory MDs, the capacity to complete a 6MWT, is not possible in a large percentage of adults with MD. Indeed, the 6MWT, although advocated as a test of functional capacity in MD,<sup>10</sup> would be impossible in 55%, 18%, 36%, and 100% of our previously described adult participants with LGMD, FSHD, BMD, and DMD, respectively.<sup>11</sup> In search of an alternative functional test, Jansen *et al.*<sup>12</sup> validated the assisted 6-minute cycle test (A6MCT) against the 6MWT in children with DMD, and found a high degree of reliability and validity.

The A6MCT, was developed in children with MD as a submaximal endurance test for those who may be wheelchair-dependent, or lack the confidence or capacity to complete timed walking tests.<sup>12</sup> The use of a motor-assisted device, set at minimal resistance and speed (7 Hz), meant that even those with limited upper limb function could contribute some active torque, however minimal, over the 6-minute period. In contrast, those children who maintained upper limb function, even in DMD, could produce an active torque, and accelerate the arm crank to produce a good discriminatory test between the most impaired of those with MD.<sup>12</sup>

At present, there are no normative A6MCT data from adults with MD. In non-ambulatory adults with MD, there are limited data on muscle function, particularly from a power-assisted task that would be sensitive to those who were most impaired. Unlike the 6MWT, which is known to be associated with knee extension strength in children with DMD,<sup>7</sup> and oxygen uptake (VO<sub>2</sub>) and lung function in cardiorespiratory

**Abbreviations:** 6MWT, 6-minute walk test; A6MCT, assisted 6-minute cycle test; ANOVA, analysis of variance; BMD, Becker muscular dystrophy; BLA, blood lactate; CTRL, control; DXA, dual-energy X-ray absorptiometry; DMD, Duchenne muscular dystrophy; FEV<sub>1</sub>, forced expiratory volume in 1 second; FSHD, facioscapulohumeral dystrophy; FVC, forced vital capacity; HR, heart rate; LGMD, limb-girdle dystrophy; MD, muscular dystrophy; PASIPD, physical activity score for individuals with physical disability; VE, pulmonary ventilation; VCO<sub>2</sub>, carbon dioxide output; VO<sub>2</sub>, oxygen uptake

**Key words:** 6-minute assisted cycle test; grip strength; lung function; muscular dystrophy; oxygen uptake

**Conflicts of Interest:** None of the authors have any conflict of interest to disclose.

**Correspondence to:** C.I. Morse; e-mail: c.morse@mmu.ac.uk

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2018 Wiley Periodicals, Inc.

Published online 17 May 2018 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mus.26146

conditions,<sup>13–15</sup> the A6MCT is yet to be presented alongside physiological parameters such as muscle size and strength and lung function in adults with MD.

Thus, the aim of this study was to: (1) compare A6MCT distance and cardiorespiratory function in adult males with MD; and (2) identify whether A6MCT is associated with physiological outcomes such as muscle strength and size and lung function.

We hypothesized that A6MCT distance would reflect previous patterns of muscular and respiratory impairment,<sup>11</sup> such that all MDs would perform lower than CTRL subjects, with DMD and BMD patients completing the least distance followed by LGMD and FSHD patients; consistent with data from the 6MWT,<sup>7</sup> we hypothesized that measures of muscle and respiratory function would correlate with A6MCT distance.

## METHODS

Forty-eight adult males volunteered for this study (Table 1), 38 of whom had previously been diagnosed with either FSHD, BMD, LGMD, or DMD; the remaining 10 had no form of MD and formed a control (CTRL) group. MD participants were classified as ambulatory or non-ambulatory, and were graded for arm and leg function using Brooke and Vignos scales, respectively,<sup>16</sup> by a chartered physiotherapist. MD participants were recruited from, and tested at, The Neuromuscular Centre (Winsford, UK). All MD participants were receiving weekly physiotherapy treatment involving passive mobility activities lasting approximately 1 hour. The CTRL subjects, free from neurological disorders and otherwise in good health, were recruited from the broader community of The Neuromuscular Centre (none were related to MD participants), or were staff/students of MMU. Control participants self-reported being sedentary, undertaking <1 hour of recreational physical activity; none of the participants were undertaking any structured or regular exercise training regimens. Two additional adult males, with DMD and BMD, were

unable to complete the testing protocol. Their reasons for withdrawal were: (1) upper limb contractures making the hand cycle range of motion impossible to complete; and (2) respiratory insufficiency to overcome the face mask used for expired gas analysis. The data presented here represent the 48 adult males who completed all procedures.

All procedures conformed to the standards set by the latest revision of the Declaration of Helsinki,<sup>17</sup> and were approved by the local ethics committee of Manchester Metropolitan University. Written informed consent was obtained from all participants before inclusion.

**Procedures.** All participants were tested on a single visit to The Neuromuscular Centre. The testing session was conducted in the following order: anthropometry; body composition assessment; lung function; grip strength; questionnaires; resting energy expenditure; and A6MCT. The same equipment was used for both population groups, with the exception of the seated scales for body mass measures in non-ambulatory MD participants.

**Anthropometric Measures.** All participant heights were calculated as point-to-point arm span (index finger, elbow, shoulder, and across midline), using a 2-m tape measure. To account for the known discrepancy between standing height and arm-span measures, a correction was applied using regression data from adult males, with the known error of making this correction of 3.5%.<sup>18</sup> Participant height is presented as this corrected value. In a sample of MD participants who could complete arm-span and standing height ( $n = 12$ ) assessment, arm span was not different from height, with an error of 7.97 cm (4.5%) and intraclass correlation (ICC) of 0.67. Estimating height from arm span within this sample, gave an error of 2.58 cm (1.4%), and ICC 0.66. In the control group, body mass was measured by digital scales (Model 873; Seca, Hamburg, Germany). MD participants were weighed in seated scales (6875; Detecto, Webb City, Missouri). The weights of slings, shoes, splints, etc., were subtracted from gross weight after weighing separately.

**Table 1.** Participants' demographics

	CTRL	FSHD	LGMD	BMD	DMD
Number of participants	10	9	11	9	9
Age (years)	29.8 (10.7)	43.9 (13.7)*	45.1 (9.2)*	37.4 (7.6)	22.7 (3.0) <sup>†‡§</sup>
Age range (years)	19–51	25–60	30–60	27–51	18–26
Stature (m)	1.79 (0.09)	1.81 (0.04)	1.76 (0.08)	1.73 (0.11)	1.72 (0.10)
Mass (kg)	76.2 (13.8)	83.5 (14.1)	94.3 (14.2)	88.8 (24.2)	74.7 (15.4)
Body fat (%)	15.2 (5.1)	22.9 (6.8)	32.4 (7.8) <sup>*,†</sup>	32.1 (9.1) <sup>*,†</sup>	32.4 (2.9) <sup>*,†</sup>
BMI (kg/m <sup>2</sup> )	24.1 (3.7)	24.0 (5.0)	29.9 (4.2) <sup>‡</sup>	31.6 (10.9)	25.5 (6.0)
Lean mass (kg)	67.4 (10.4)	62.9 (12.9)	63.8 (7.6)	59.0 (12.3)	50.7 (10.0)*
PASIPD	—	11.7 (9.0)	14.8 (11.6)	5.7 (4.8)	4.1 (4.8) <sup>§</sup>
Biceps thickness (mm)	39.6 (5.0)	31.2 (10.1)	30.7 (6.7)	26.7 (9.8)*	26.4 (8.9)*
Ambulatory (%)	100	89	36	22	0
Brooke scale	—	1 (1–3)	3 (1–5) <sup>†</sup>	3 (2–4) <sup>†</sup>	6 (4–6) <sup>†‡§</sup>
Vignos scale	—	2 (1–9)	9 (2–9) <sup>†</sup>	9 (4–9) <sup>†</sup>	9 (9) <sup>†</sup>

Data are presented as mean (SD), except for Brooke and Vignos scales, which are presented as mean (range). CTRL, control; LGMD, limb girdle muscular dystrophy; FSHD, fascioscapulohumeral; BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; BMI, body mass index; PASIPD, Physical Activity Score for Individuals with Physical Disability.

\*Significant difference vs. CTRL ( $P < 0.05$ ).

<sup>†</sup>Significant difference vs. FSHD ( $P < 0.05$ ).

<sup>‡</sup>Significant difference vs. BMD ( $P < 0.05$ ).

<sup>§</sup>Significant difference vs. LGMD ( $P < 0.05$ ).

**Body Composition.** Fat mass and fat-free mass (FFM) were measured using a bioelectrical impedance (BIA) device (Model 1500; Bodystat, Isle of Man, UK) with adhesive electrodes placed on the right hand (between styloid process of ulna and radius) and foot (between the medial and lateral malleoli). Body composition, FFM, and fat mass were determined using the proprietary equations within the BIA device. BIA has previously been used within DMD populations<sup>19</sup> and has been shown to be valid and reliable compared with dual-energy X-ray absorptiometry (DXA) in both normal and overweight populations.<sup>20,21</sup> In addition, the portability of the BIA makes it a viable alternative to DXA in subjects with limited mobility.<sup>22</sup>

**Biceps Thickness.** Biceps thickness was measured in all participants, consistent with previously established methods.<sup>23</sup> Participants were seated with their dominant arm hanging, relaxed at their side. The medial and lateral boundaries of the biceps were identified in the transverse plane using B-mode ultrasound (MyLab Gamma; Esaote, Reading, Berks., UK) with a 7.5-MHz linear-array probe. Thickness measurements (distance between the superficial and deep aponeurosis) were taken on the mid-sagittal line of the biceps at 60% of the upper arm length (the distance from the acromion process of the scapular to the lateral epicondyle of the humerus). Three measurements were made at the proximal, middle, and end of the displayed image, from 3 images of the biceps. The biceps thickness presented is the average of these measures.

**Lung Function.** Forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV<sub>1</sub>) were measured using an electronic spirometer (Pneumotrac; Vitalograph, Bucks., UK). All participants performed the measurements seated and wearing a nose clip. The best performance of 3 trials is reported. The validity of the electronic vitalograph has been established in healthy and clinical populations.<sup>24</sup>

**Grip Strength.** A digital handgrip dynamometer (Jamar Plus; Patterson Medical, Warrenville, Illinois) was used to measure grip strength. Of those participants who could produce a measureable grip force (2 DMD participants produced no grip strength), 3 maximal attempts were made. Participants performed all 3 of the 5-s maximal grip efforts while in a seated position using the dominant hand and with the arm in a relaxed position at their side. All 3 tests were separated by 1 minute, and the peak reading was recorded. Test-retest reliability has been reported as high, with low measurement error.<sup>25</sup>

**Physical Activity Questionnaire.** MD participants completed a disability-specific physical activity questionnaire [i.e., the Physical Activity Score for Individuals with Physical Disabilities (PASIPD) questionnaire].<sup>26</sup> The PASIPD questionnaire encompasses a lower activity threshold and has been validated as a reliable outcome measure for physical activity in individuals with disability.<sup>27</sup>

**Expired Gas Analysis.** Expired gas analysis was performed at rest and during the A6MCT using a face mask and volume sensor assembly (Metalyser; Cortex, Leipzig, Germany). Fractional concentrations of oxygen and carbon dioxide were measured via a capillary line from the face mask. The gas analyzer and volume transducer were corrected for ambient conditions (pressure and temperature)

and calibrated before each test. All participants were seated during resting and exercising measurements.

**Resting Measures.** Resting measures were recorded for 1 minute after 20 minutes of seated rest with the face mask attached. This duration was chosen as it allows a sufficient plateau in respiration to acquire reliable data.<sup>28</sup> Outcome measures for the expired gas analysis are described in what follows.

**A6MCT.** The A6MCT was conducted in accordance with the protocol of Jansen *et al.*<sup>12</sup>; however, only upper limb testing was performed as this allowed a wider range of participants to be recruited. Participants performed the A6MCT using a motor-assisted hand bike mobility trainer (MOTomed; Viva2, Reck, Germany), with a zero load speed of 7 rpm, consistent with previously adopted methods.<sup>12</sup> Participants were instructed to cycle as fast as possible for 6 minutes, while receiving verbal encouragement. Those MD participants who were unable to produce an active torque throughout the 6 minutes were encouraged to rest, allowing the hand bike to passively move their arms, until they were able to continue producing an active torque. The primary outcome measure of the A6MCT was cycle distance. Heart rate (HR) was monitored by short-range telemetry (Polar, Kempele, Finland) throughout the exercise test every 5 seconds and is presented averaged over each minute of exercise.

Blood lactate was assessed using fingertip capillary samples (Accutrend Plus; Roche Diagnostics, Ltd., London, UK) at 5 minutes before (BLA<sub>pre</sub>), and immediately after A6MCT (BLA<sub>post</sub>).

One-minute averages for rest, and for each minute of the A6MCT, were taken from the breath-by-breath samples and are presented as standard temperature, pressure, and dry volumes, where relevant. Reported outcome measures include: pulmonary ventilation (VE); oxygen uptake (VO<sub>2</sub>); carbon dioxide output (VCO<sub>2</sub>); and respiratory quotient. VO<sub>2</sub> and VCO<sub>2</sub> are presented relative to body mass.

**Statistics.** All analyses were performed using IBM SPSS version 24 software (IBM SPSS, Armonk, New York). Parametric assumptions of normal distribution were confirmed using the Shapiro-Wilk test ( $P > 0.05$ ) in all dependent variables, except for total A6MCT distance ( $P < 0.05$ ). Differences between the groups were analyzed using a one-way analysis of variance (ANOVA) with either Tukey or Games–Howell *post-hoc* test, depending on whether variance was homogeneous (Levene's test,  $P > 0.05$ ) or non-homogeneous (Levene's test,  $P < 0.05$ ), respectively. Variables that violated normal distribution were compared between groups using the Kruskal–Wallis test, with *post-hoc* Mann–Whitney *U*-test pairwise comparisons when appropriate.

Where comparisons were made at 1-minute intervals over the A6MCT, a  $3 \times 6$  ANOVA was performed. Tukey *post-hoc* test was conducted whenever group differences were observed.

With all *post-hoc* comparisons, unless otherwise stated, differences are indicated in one direction of comparison; that is, if a group performed “worse” than any other, the *P* value is reported, or denoted with the relevant superscript.

Correlations were performed using grouped data (pooled FSHD, LGMD, and BMD) in the conditions that showed no between-group differences for dependent variables. The Pearson correlation was performed because these data were normally distributed.



**Table 2.** Muscle, respiratory, and exercise performance

	CTRL	FSHD	LGMD	BMD	DMD
Grip strength (kg)	44.9 (9.9)	25.9 (15.5)*	17.8 (9.8)*	9.7 (6.3)* <sup>†</sup>	2.0 (2.6)* <sup>†‡</sup>
FEV <sub>1</sub> (L)	4.12 (0.70)	2.79 (0.70)*	2.28 (0.79)*	3.21 (1.15)	0.88 (0.54)* <sup>†‡§</sup>
FVC (L)	5.14 (0.95)	3.49 (0.92)*	2.95 (1.07)*	3.80 (1.42)*	1.13 (0.68)* <sup>†‡§</sup>
FEV <sub>1</sub> /FVC	0.81 (0.08)	0.81 (0.10)	0.78 (0.08)	0.85 (0.04)	0.82 (0.19)
A6MCT distance (km)	3.53 (0.11)	2.54 (0.44)*	2.39 (0.82)*	2.13 (0.80)*	0.45 (0.54)* <sup>†‡§</sup>
BLa <sub>pre</sub> (mmol/L)	1.99 (0.90)	2.22 (0.70)	2.15 (0.79)	2.44 (1.80)	1.93 (0.58)
BLa <sub>post</sub> (mmol/L)	8.40 (3.02)	5.02 (2.10)*	5.03 (2.49)*	3.71 (1.38)*	2.17 (0.75)* <sup>†§</sup>

Data are presented as mean (SD). CTRL, control; LGMD, limb girdle muscular dystrophy; FSHD, fascioscapulohumeral; BMD, Becker muscular dystrophy; DMD, Duchenne muscular dystrophy; FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; BLa, blood lactate; A6MCT, assisted 6-minute cycle test.

\*Significant difference vs. CTRL ( $P < 0.05$ ).

<sup>†</sup>Significant difference vs. FSHD ( $P < 0.05$ ).

<sup>‡</sup>Significant difference vs. BMD ( $P < 0.05$ ).

<sup>§</sup>Significant difference vs. LGMD ( $P < 0.05$ ).

All data are reported as mean (standard deviation). Statistically significant differences and/or associations were accepted at  $\alpha \leq 0.05$ .

## RESULTS

### Demographic, Anthropometric, and Body Composition Measures.

As shown in Table 1, DMD participants were younger than LGMD, BMD, and FSHD participants. Compared with CTRL, LGMD and FSHD were older. DMD and BMD showed no age difference compared with CTRL. There were no differences in stature or body mass between groups. LGMD, BMD, and DMD had higher body fat percentage than FSHD and control participants. Lean mass was lower in DMD than CTRL subjects, with no lean mass differences observed between other groups.

**Biceps Thickness.** Compared with CTRL, DMD and BMD had smaller biceps thickness. There was no difference between MD populations for biceps thickness. LGMD and FSHD biceps thicknesses were not different from those of CTRL (Table 1).

**Physical Activity.** Physical activity was lower in DMD than LGMD (Table 1); there was no difference between the other MD groups.

**Lung Function.** Compared with CTRL, FEV<sub>1</sub> was lower in FSHD, LGMD, and DMD. Compared with the other MD groups, DMD had a lower FEV<sub>1</sub>. FVC was lower than that for CTRL in all MD groups. DMD had a lower FVC than the other MD groups (Table 2).

**Grip Strength.** Compared with CTRL, grip strength was less in all MD groups. DMD had lower grip strength than LGMD and FSHD (Table 2).

**A6MCT Distance.** A6MCT distance was shorter in FSHD, LGMD, BMD, and DMD when compared with CTRL. The DMD A6MCT distance was shorter when compared with FSHD, LGMD, and BMD.

There was no difference in A6MCT distance between BMD, LGMD, and FSHD (Table 2).

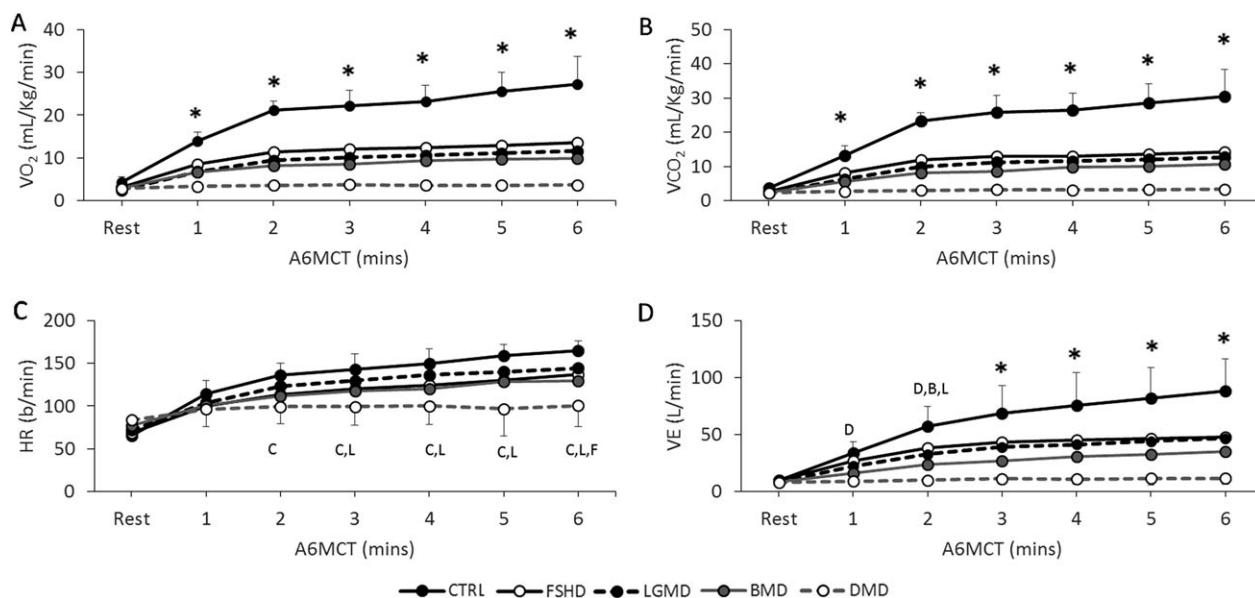
**A6MCT VO<sub>2</sub>.** Throughout the A6MCT, VO<sub>2</sub> in DMD, BMD, LGMD, and FSHD was lower than in CTRL. VO<sub>2</sub> during the A6MCT was lower in DMD compared with other MD groups. There was no difference in VO<sub>2</sub> during the A6MCT at any time-point between BMD, FSHD, or LGMD (Fig. 1A).

**A6MCT VCO<sub>2</sub>.** Throughout the A6MCT, VCO<sub>2</sub> was lower than CTRL than in DMD, BMD, LGMD, and FSHD. Compared with the other MDs, DMD had lower VCO<sub>2</sub> throughout the A6MCT. There was no difference in VCO<sub>2</sub> during the A6MCT between BMD, FSHD, and LGMD (Fig. 1B).

**A6MCT HR.** Throughout the A6MCT, DMD had a lower HR than CTRL at minutes 2–6. In addition, DMD HR during the A6MCT was lower than LGMD at minutes 3–6, and lower than LGMD at minute 6. BMD had a lower HR than CTRL at minute 6. There was no difference in HR during the A6MCT between LGMD, BMD, and FSHD (Fig. 1C).

**A6MCT VE.** Compared with CTRL, VE during the A6MCT was lower in DMD at all time-points. In BMD and LGMD, VE was lower than in CTRL at minutes 2–6 of the A6MCT. FSHD VE was lower than in CTRL at minutes 3–6 of the A6MCT. Within the MD participants, DMD VE was lower than in BMD at minutes 1 and 4, and lower than that in LGMD and FSHD at all time-points of the A6MCT (Fig. 1D).

**A6MCT BLa.** Pre-A6MCT BLa was not different between groups (Table 2). After A6MCT, BLa was lower in all MD groups compared with CTRL. Compared with FSHD and LGMD, BLa<sub>post</sub> was lower in DMD. Within-group changes in BLa from pre to post were significant in all groups, except from



**FIGURE 1.** Cardiorespiratory measures recorded during the assisted 6-minute cycle test. **(A)** Oxygen uptake ( $VO_2$ ). **(B)** Carbon dioxide output ( $VCO_2$ ). **(C)** Heart rate (HR). **(D)** Ventilation (VE). Asterisk: significant difference vs. all other groups ( $P < 0.05$ ); C: significant difference from CTRL; F: significant difference from FSMD; B: significant difference from BMD; L: significant difference from LGMD (all group differences are  $P < 0.05$ ). For clarity, significant differences from CTRL only are shown on the ventilation image. Data are presented as mean (SD).

DMD, where there was no change in  $BLa_{pre}$  to  $BLa_{post}$ .

**Correlations.** Within the pooled MD participants (BMD, FSMD, and LGMD,  $n = 29$ ), A6MCT distance was correlated with handgrip strength ( $r = 0.609$ ,  $P < 0.05$ ),  $FEV_1$  ( $r = 0.455$ ,  $P < 0.05$ ), FVC ( $r = 0.518$ ,  $P < 0.05$ ), body fat percentage ( $r = 0.571$ ,  $P < 0.05$ ), and  $BLa_{post}$  ( $r = 0.437$ ,  $P < 0.05$ ). In terms of cardiorespiratory measures during A6MCT, throughout the final minute of exercise (others are omitted for clarity), the A6MCT distance correlated with  $VO_2$  ( $r = 0.638$ ,  $P < 0.05$ ),  $VCO_2$  ( $r = 0.636$ ,  $P < 0.05$ ), HR ( $r = 0.472$ ,  $P < 0.05$ ), and VE ( $r = 0.708$ ,  $P < 0.05$ ). No correlations were observed for A6MCT distance in other measures, including PASIPD, body mass, body mass index, stature, lean mass, and biceps thickness.

In CTRL, A6MCT distance correlated with handgrip strength ( $r = 0.811$ ) and biceps thickness ( $r = 0.634$ ). No other correlations were observed for A6MCT distance in CTRL.

## DISCUSSION

The main finding in this study is in agreement with our hypothesis that A6MCT distance was lower in adults with MD compared with CTRLs; in addition, adult males with DMD had a shorter A6MCT distance than those with FSMD, BMD, and LGMD. Within FSMD, BMD, and LGMD participants, the variance in A6MCT distance could be attributed to lung function ( $FEV_1$  and FVC); grip strength

(consistent with our hypothesis); and cardiorespiratory measures of  $VO_2$ ,  $VCO_2$ , HR, and VE.

The A6MCT has previously been utilized in boys with DMD.<sup>12,29,30</sup> Although different hand bikes were used, and earlier data were presented as total revolutions,<sup>12,29</sup> when estimated based on the distance achieved through 7-rpm passive cycling, our present findings with adults (CTRL = 687–781 revolutions) are similar to those presented previously (CTRL boys ~800 revolutions). Interestingly, boys with DMD previously achieved ~350 revolutions,<sup>12</sup> whereas the estimated value in the present adult DMD participants was lower (94 revolutions), consistent with the progressive nature of DMD.

The lower A6MCT distance in the DMD participants, compared with FSMD, BMD, and LGMD, may be attributed to the lower grip strength and lung function, and to lower HR,  $VO_2$ ,  $VCO_2$ , and VE throughout the A6MCT. Although speculative based on the present data, grip strength likely reflects the recruitable muscle mass available to produce cycle torque, and lung function reflects the ability of the respiratory system to respond to the demands of exercise stress. In DMD, there was minimal grip strength and impaired lung function, and therefore lower A6MCT distance. Further evidence for the inability of adults with DMD to respond to an exercise demand was supported by the lack of any metabolic byproducts, with no change from  $BLa_{pre}$  to  $BLa_{post}$ .

The correlations in the FSMD, BMD, and LGMD participants and physiological parameters suggest

that the A6MCT is a good measure of neuromuscular and cardiorespiratory function. It should be noted that those measures with the highest correlation (grip strength, VE, and VO<sub>2</sub>) could only explain ~40% of the variance in A6MCT. However, the present correlations between the A6MCT and physiological measures are similar to those observed previously for the 6MWT and knee extension myometry.<sup>7</sup> Indeed, based on the previously reported correlations between myometry and walking tasks ( $r = 0.4\text{--}0.7$ ), the 6MWT was advocated in MD as a valid single measure of “cardiac, respiratory, circulatory, and muscular capacity.”<sup>7</sup>

In terms of its appropriateness in adults with MD, none of the present adults with DMD and only 48% of the other adult MD participants could start a 6MWT. Similarly, validation testing of the A6MCT (e.g., VO<sub>2peak</sub>) could only be completed in some of these participants, and, beyond the grip strength measures presented here, there are presently no data comparing tests of upper limb function with A6MCT distance. Previously, the A6MCT and 6MWT were found to be in agreement ( $r = 0.65$ ), with high retest reliability for the A6MCT (ICC = 0.89, 95% CI 0.76–0.95).<sup>12</sup> Therefore, the A6MCT previously validated in children with MD is an exercise test that can be completed by adults with MD who are non-ambulatory. Furthermore, in FSHD, BMD, and LGMD, it represents a single measure that correlates well with grip strength and lung function.

We observed no correlations between biceps thickness and lean mass and A6MCT distance in the pooled FSHD, LGMD, and BMD participants; this is surprising as grip strength correlated with A6MCT distance. The assessment of lean mass and biceps thickness may therefore not reflect the recruitable musculature in the MD participants. It is well established that there is substantial remodeling of muscle mass in MD,<sup>31</sup> with infiltration of non-contractile material, such that “pseudohypertrophy” is reported in some MD muscles.<sup>32</sup> It is likely that using more stringent measures of assessment (e.g., MRI or DXA), particularly in the upper limb, may have reflected more closely the muscle mass available for performing the A6MCT, as evidenced by the present grip strength results. Within the CTRL group, where one would assume the infiltration of non-contractile material is lower than in those with MD, A6MCT distance correlated with biceps thickness ( $r = 0.634$ ). The associations in the MD participants between A6MCT distance, grip strength, VO<sub>2</sub>, and BLA<sub>post</sub> all suggest that recruitable muscle mass in the upper limb contributes to A6MCT, despite not being related to biceps thickness or lean mass in the present study. Although the use of ultrasonography may therefore be considered a limitation in our study, due to lower limb contractures and the lack

of mobility in some of our more impaired MD participants, MRI or DXA would have precluded their inclusion.

We found no between group differences in certain outcome measures (e.g. grip strength), we acknowledge that, in this study, but we acknowledge that, due to the heterogeneity (and associated measurement variance) of the participants within each category, a greater number of participants would have been beneficial. The paucity of data from adult MD populations is such that the only previous data on the primary outcome measure (A6MCT) is in children with DMD vs. CTRL,<sup>12</sup> where the effect size ( $d = 2.42$ ) results in a recommended group size of 2 (G\*Power, Kiel, Germany). However, we do acknowledge a limitation in the present study that MD group differences may not be present in some outcome measures due to type 2 error associated with high variance. An example of this can be seen for grip strength, where the coefficient of variation was 55%–65% in LGMD, FSHD, and BMD, compared with 22% in CTRL. This heterogeneity of outcome measures is to be expected within MD, and is likely to have contributed to the correlational findings in the present study.

In conclusion, adult males with MD had a shorter A6MCT distance than adult controls. Furthermore, adult males with DMD had lower A6MCT than those with FSHD, LGMD, and BMD. In adult males with BMD, LGMD, and FSHD, the A6MCT variance was attributable to grip strength and lung function. The A6MCT can therefore be considered an inclusive and physiologically meaningful test of physical function for adults with MD.

**Ethical Publication Statement:** We (the authors) confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

## REFERENCES

1. Emery AE. The muscular dystrophies. *Lancet* 2002;359:687–695.
2. Bushby K. The limb-girdle muscular dystrophies: diagnostic guidelines. *Eur J Paediatr Neurol* 1999;3:53–58.
3. Mercuri E, Muntoni F. Muscular dystrophies. *Lancet* 2013;381:845–860.
4. Tawil R, Kissel JT, Heatwole C, Pandya S, Gronseth G, Benatar M. Evidence-based guideline summary: evaluation, diagnosis, and management of facioscapulohumeral muscular dystrophy. *Neurology* 2015;85:357–364.
5. Lue YJ, Su C-Y, Yang R-C, Su W-L, Lu Y-M, Lin R-F, *et al.* Development and validation of a muscular dystrophy-specific functional rating scale. *Clin Rehabil* 2006;20:804–817.
6. Eichinger K, Heatwole C, Heininger S, Stinson N, Matichak Stock C, Grosman C, *et al.* Validity of the 6 minute walk test in facioscapulohumeral muscular dystrophy. *Muscle Nerve* 2017;55:333–337.
7. McDonald CM, Henricson EK, Abrisch RT, Florence J, Eagle M, Gappmaier E, *et al.* The 6-minute walk test and other clinical endpoints in Duchenne muscular dystrophy: reliability, concurrent validity, and minimal clinically important differences from a multicenter study. *Muscle Nerve* 2013;48:357–368.
8. Kierkegaard M, Tollbäck A. Reliability and feasibility of the six minute walk test in subjects with myotonic dystrophy. *Neuromuscul Disord* 2007;17:943–949.
9. Graham JE, Ostir GV, Fisher SR, Ottenbacher KJ. Assessing walking speed in clinical research: a systematic review. *J Eval Clin Pract* 2008;14:552–562.

10. Lynn S, Aartsma-Rus A, Bushby K, Furlong P, Goemans N, De Luca A, *et al.* Measuring clinical effectiveness of medicinal products for the treatment of Duchenne muscular dystrophy. *Neuromuscul Disord* 2015;25:96–105.
11. Morse CI, Smith J, Denny A, Tweedale J, Searle ND, Winwood K, *et al.* Bone health measured using quantitative ultrasonography in adult males with muscular dystrophy. *J Musculoskel Neuron Interact* 2016;16:339–347.
12. Jansen M, De Jong M, Coes HM, Eggermont F, Van Alfen N, De Groot IJ. The assisted 6-minute cycling test to assess endurance in children with a neuromuscular disorder. *Muscle Nerve* 2012;46:520–530.
13. Ross RM, Murthy JN, Wollak ID, Jackson AS. The six minute walk test accurately estimates mean peak oxygen uptake. *BMC Pulm Med* 2010;10:31.
14. Cahalin LP, Mathier MA, Semigran MJ, Dec GW, DiSalvo TG. The six-minute walk test predicts peak oxygen uptake and survival in patients with advanced heart failure. *Chest* 1996;110:325–332.
15. Celli B, Tetzlaff K, Criner G, Polkey MI, Sciurba F, Casaburi R, *et al.* The 6-minute-walk distance test as a chronic obstructive pulmonary disease stratification tool. Insights from the COPD Biomarker Qualification Consortium. *American journal of respiratory and critical care medicine* 2016;194(12):1483–1493.
16. Lue YJ, Lin R-F, Chen S-S, Lu Y-M. Measurement of the functional status of patients with different types of muscular dystrophy. *Kaohsiung J Med Sci* 2009;25:325–333.
17. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013;310:2191–2194.
18. Reeves S, Varakamin C, Henry C. The relationship between arm-span measurement and height with special reference to gender and ethnicity. *Eur J Clin Nutr* 1996;50:398–400.
19. Garby L, Lammert O. Between-subjects variation in energy expenditure: estimation of the effect of variation in organ size. *Eur J Clin Nutr* 1994;48:376–378.
20. Sun G, French CR, Martin GR, Younghusband B, Green RC, Xie Y-G, *et al.* Comparison of multifrequency bioelectrical impedance analysis with dual-energy X-ray absorptiometry for assessment of percentage body fat in a large, healthy population. *Am J Clin Nutr* 2005;81:74–78.
21. Okasora K, Takaya R, Tokuda M, Fukunaga Y, Oguni T, Tanaka H, *et al.* Comparison of bioelectrical impedance analysis and dual energy X-ray absorptiometry for assessment of body composition in children. *Pediatr Int* 1999;41:121–125.
22. McDonald CM, Carter GT, Abresch RT, Widman L, Styne DM, Warden N, *et al.* Body composition and water compartment measurements in boys with Duchenne muscular dystrophy. *Am J Phys Med Rehabil* 2005;84:483–491.
23. Miyatani M, Kanehisa H, Ito M, Kawakami Y, Fukunaga T. The accuracy of volume estimates using ultrasound muscle thickness measurements in different muscle groups. *Eur J Appl Physiol* 2004;91:264–272.
24. Fonseca JA, Costa-Pereira A, Delgado L, Silva LN, Magalhaes M, Castel-Branco MG, *et al.* Pulmonary function electronic monitoring devices: a randomized agreement study. *Chest* 2005;128:1258–1265.
25. Shechtman O, Gestewitz L, Kimble C. Reliability and validity of the DynEx dynamometer. *J Hand Ther* 2005;18:339–347.
26. Washburn RA, Zhu W, McAuley E, Frogley M, Figoni SF. The physical activity scale for individuals with physical disabilities: development and evaluation. *Arch Phys Med Rehabil* 2002;83:193–200.
27. van der Ploeg H, Streppel K, van der Beek A, Van der Woude L, Vollenbroek-Hutten M, van Mechelen W. The physical activity scale for individuals with physical disabilities: test-retest reliability and comparison with two accelerometers. *Arch Phys Med Rehabil* 2007;4:96–100.
28. Jacques MF, Orme P, Smith J, Morse CI. Resting energy expenditure in Becker's muscle dystrophy. *PLoS One* (to be published).
29. Dirks I, Koene S, Verbruggen R, Smeitink JA, Jansen M, De Groot IJ. Assisted 6-minute cycling test: An exploratory study in children. *Muscle Nerve* 2016;54:232–238.
30. Jansen M, van Alfen N, Geurts AC, de Groot IJ. Assisted bicycle training delays functional deterioration in boys with Duchenne muscular dystrophy: the randomized controlled trial “no use is disuse.” *Neuro-rehabil Neural Repair* 2013;27:816–827.
31. Wokke B, van den Bergen J, Versluis M, Niks E, Milles J, Webb A, *et al.* Quantitative MRI and strength measurements in the assessment of muscle quality in Duchenne muscular dystrophy. *Neuromuscul Disord* 2014;24:409–416.
32. Vohra RS, Lott D, Mathur S, Senesac C, Deol J, Germain S, *et al.* magnetic resonance assessment of hypertrophic and pseudo-hypertrophic changes in lower leg muscles of boys with Duchenne muscular dystrophy and their relationship to functional measurements. *PLoS One* 2015;10:e0128915.